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Review

Immune Reconstitution after Haploidentical Hematopoietic Stem Cell Transplantation

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Haploidentical hematopoietic stem cell transplantation (HSCT) offers the benefits of rapid and nearly universal donor availability and has been accepted worldwide as an alternative treatment for patients with hematologic malignancies who do not have a completely HLA-matched sibling or who require urgent transplantation. Unfortunately, serious infections and leukemia relapse resulting from slow immune reconstitution remain the 2 most frequent causes of mortality in patients undergoing haploidentical HSCT, particularly in those receiving extensively T cell–depleted megadose $CD34^+$ allografts. This review summarizes advances in immune recovery after haploidentical HSCT, focusing on the immune subsets likely to have the greatest impact on clinical outcomes. The progress made in accelerating immune reconstitution using different strategies after haploidentical HSCT is also discussed. It is our belief that a predictive immune subset–guided strategy to improve immune recovery might represent a future clinical direction.

INTRODUCTION

Haploidentical hematopoietic stem cell (HSC) transplantation (HSCT) is available for nearly all patients and has no search or acquisition costs [1-9]. Over the past decade, many haploidentical transplantation protocols, including T cell–replete and T cell–depleted (TCD) haplotype HSCT, depending on whether or not allografts have been engineered in vitro, have demonstrated promising clinical outcomes [4,8,9]. Unfortunately, serious infections and disease relapse resulting from delayed immune reconstitution remain the 2 most frequent causes of mortality after allogeneic HSCT, particularly in patients who received extensively TCD $CD34^+$ cell megadose allografts [10-15]. Advances in the understanding of immune recovery profiles in haploidentical recipients [8,16-47], along with new methods of modifying donor T cells [48-50] and natural killer (NK) cells [51], have made it possible to establish new strategies to improve post-transplantation immunologic reconstitution [9,52-58].

In this review, we summarize advances in immune recovery after haploidentical HSCT, focusing on the recovered immune subsets likely to have the greatest impact on clinical outcomes [18,23,33,42,45,59-63]. We compare the differences in immune reconstitution between haploidentical transplantation and other transplantation strategies, including HLA-identical sibling donor transplantation, HLA-matched unrelated donor transplantation, and umbilical cord blood transplantation. In addition, we discuss recent advances in the enhancement of immune reconstitution after haploidentical HSCT [64-70].

KINETICS OF IMMUNE RECOVERY AFTER HAPLOIDENTICAL HSCT

Different immune cell subgroups recover at different rates after haploidentical HSCT (Table 1). The conditioning regimen is followed by a "neutropenic" phase that lasts until neutrophils reconstitute, at a median of approximately 11 to 12 days after TCD HSCT with high doses of $CD34^+$ cells [8,38], approximately 15 days after unmanipulated haploidentical steady-state bone marrow (BM) allografts, approximately 21 days after granulocyte colony-stimulating factor (G-CSF)-primed BM allografts, and approximately 13 days after G-CSF–stimulated blood and BM allografts [2,7,71]. The recovery of neutrophil function (eg, chemotaxis, phagocytosis, superoxide production, killing of bacteria) in haploidentical settings remains poorly understood, however.

The rapid recovery of NK cells after haploidentical transplantation is based on an expansion of the cytokine-producing $CD56^{bright}$ NK cell subsets in both T cell–replete and TCD settings [16,17,24,41]. Although the absolute number of overall NK cells usually recovers to the donor's level by day 30 post-HSCT, recovery may be delayed by the development of graft-versus-host disease (GVHD) [8,24]. The function of NK cell recovery is regulated by the balance of activating and inhibitory signals transmitted by different cell surface receptors. Thus, the expression of activating NK receptors (NKRs), such as NKP46, NKP44, NKP30, and NKG2D, as well as inhibiting NKRs, such as CD158a, CD158b, CD158e, and NKG2A, is essential for NK cell activation.

After haploidentical HSCT, the overall expression of activating NKRs and inhibitory NKRs is reduced, whereas CD94/NKG2A expression is increased. NKG2A recovery is inversely correlated with CD158 recovery in the year after HSCT. This altered phenotype includes more $CD56^{bright}$ cells, fewer $CD56^{dim}$ NK cells, and altered CD94/NKGA expression. Activation or inhibition of NKR expression during early reconstitution is associated with lower levels of in vitro NK

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cytotoxicity after haploidentical HSCT. The reconstitution of killer cell immunoglobulin-like receptors (KIRs) is influenced by many factors, including the conditioning regimen, level of T cell depletion, and the use of immune suppression after transplantation, as recently reviewed by Zhao et al. [72]. Monocyte engraftment is rapid, with normal values on day +15 after unmanipulated haploidentical blood and BM transplantation [25]. In HLA-identical HSCT settings, the absolute monocyte counts at day +30 (>300 cells/μL) are strongly associated with improved survival [73,74], whereas no association between monocyte recovery and outcome after haploidentical HSCT has been reported.

Dendritic cells (DCs) are highly important antigen-presenting cells with central roles in initiating and modulating immune responses. Human peripheral blood DCs are divided into 2 major subsets: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). mDCs are further subdivided into 2 subsets: T helper cell (Th) 1-promoting mDCs (mDC1s) and Th2-promoting mDCs (mDC2s) [75]. Our preliminary data showed extremely low mDC1, mDC2, and PDC counts at 1-3 months post-HSCT, which recovered to normal values by 1 year after unmanipulated haploidentical blood and BM transplantation [25]. A gradual increase in the DC population after transplantation was also reported in pediatric patients with acute leukemia who received CD3/CD19-depleted grafts from haploidentical donors [41]. The early delayed immune reconstitution of DCs may contribute in part to an increased rate of postengraftment bacterial and fungal infections [76], although little is known about the recovery of DC function after haploidentical transplantation [25,41].

Invariant NK T (iNKT) cells are a specialized subset of T cells that use their T cell receptors (TCRs) to recognize self and foreign lipids presented by CD1d as cognate antigens. These cells can have protective or harmful roles in many pathological states, including microbial infection, autoimmune disease, allergic disease, and cancer [77]. In pediatric patients, de Lalla et al. [33] observed that iNKT recovered slowly after CD34-selected haploidentical HSCT and reached normal reference values by 18 months post-transplantation. Their data also suggest that the frequency of iNKT cells is significantly correlated with the remission state after haploidentical HSCT. In HLA-identical transplantation settings, Chaidos et al. [78] reported that CD4(-) iNKT cell dose was the sole graft parameter predictive of clinically significant acute GVHD. This interesting finding awaits confirmation in haploidentical HSCT settings.

Detailed analyses of adaptive immune reconstitution have been performed by researchers at various transplantation centers [16,17,22,25-27,29,32,33,38,41,45,79-81]. After CD34-selected haplodentical HSCT, mean (±standard deviation) $CD4^+$ cell counts ranged from 100 ± 40/L to 200 ± 20/L at 10 months post-transplantation, and rose thereafter. The mean $CD8^+$ cell count reached 230 ± 80/L on day +60, followed by a steady rise to 570 ± 125/L by day +300. The mean $CD16^+$ NK cell count reached 400/L stably by day +30 [38]. The recovered NK cells may enhance the graft-versus-leukemia effect [38-82]. A German group used negative selection of CD3/CD19 cells to retain NK cells, monocytes, and DCs in haploidentical allografts and found delayed T cell reconstitution, with a median of 369 $CD3^+$ cells/μL, 177 $CD4^+$ cells/μL, and 193 $CD8^+$ cells/μL on day +400 [8,83]. In our unmanipulated haploidentical blood and BM transplantation protocol, a median absolute number of 277 $CD4^+$ T cells/μL and 884 $CD8^+$ T cells/μL were recovered at 1 year post-transplantation [25]. Immune recovery after unmanipulated haploidentical HSCT appears to be more rapid than CD34-selected haploidentical transplantation; however, the 2 transplantation methods are not strictly comparable [17,25,38,39].

The expansion of memory T cells (especially $CD4^+$ memory T cells, which reconstitute later than $CD8^+$ memory T cells and rely more on thymic production of naive T cells) results in a significant inversion of the CD4/CD8 ratio up to 1 year after transplantation [25,39,41]. In contrast to most previously reported results [25,27,36,38,39,41], no significant inversion of the CD4/CD8 ratio was observed after haploidentical HSCT using reduced-intensity conditioning and CD3/CD19-depleted grafts [17]. In the TCD haploidentical HSCT protocol [45], the main lymphocyte populations (including $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, and B cells) and the CD4/CD8 ratio reconstituted to age-matched control levels by 4 to 6 years post-transplantation. Similar kinetics in B cell recovery have been reported by our group and others [17,25,41,84], with absolute numbers of B cells increasing gradually and reaching normal levels more than 1 year after HSCT.

Regarding the reconstutiton of T cell function, an interesting finding is that the ability of T cells to secrete IFN and IL-4 recovers to normal level by day +30 post-HSCT in patients without acute GVHD, although TCR rearrangement excision DNA circle (TREC) levels remain low for 12 to 24 months after unmanipulated haploidentical HSCT [29]. At 4 to 6 years after TCD haploidentical HSCT, recipients demonstrate higher proportions of $CD31^+$ naïve $CD4^+$ T cells compared with donors [17,45]. The signal-joint TCR excision circle (sjTREC) levels in the recipients tend to be higher than the levels in donors, which are similar to those seen in age-matched control subjects.

The foregoing observations suggest that long-term T cell reconstitution is critically influenced by de novo T cell production, reflecting thymopoiesis as a central mechanism. A skewed and restricted distribution of TCR repertoires has been observed at less than 180 days post-transplantation [17]. Azevedo et al. [45] reported that at 4-6 years post-transplantation, patient $CD4^+$ T cells and $CD8^+$ T cells exhibit a diverse T cell repertoire and reconstitute a diverse regulatory T cell (Treg) pool through a mechanism involving de novo thymic production. Their findings provide strong evidence that a normal immune system can be reconstituted after haploidentical HSCT; however, the small number of patients in their study precluded from a subgroup analysis to identify the factors influencing immune reconstitution. In addition, the function of T cells in unmanipulated haploidentical HSCT remains to be investigated in depth [2,71,85-88].

More recently, Ciurea et al. [36] demonstrated that compared with patients who underwent TCD haploidentical HSCT, patients who received T cell–replete haploidentical allografts exhibit better immune reconstitution of T cell subsets, including $CD4^+$ T cells, $CD8^+$ T cells, naive T cells, and memory T cells, during the first 6 months after transplantation (Table 1). The authors suggested that improved immune reconstitution might contribute to the improved early outcomes observed with the use of T cell–replete grafts [36]. Thus, a prospective study to compare immune recovery between TCD and T cell–replete haploidentical approaches is warranted.

Overall, the first 90 days after haploidentical HSCT are characterized by persistent $CD4^+$ and $CD4^+$ naive T cells, B cell lymphopenia, and low thymic function, which render patients especially susceptible to viral and fungal infections

Table 1
Immune Reconstitution in Hapolidentical Transplantation and MSDT, MUDT, and Umbilical Cord Blood Transplantation

	Federmann et al. [17]	Chang et al. [25]		Ciurea et al. [36]		Pérez-Martínez et al. [41]		Kesserwan et al. [43]*	Dominietto A et al. [93]*			
		MSDT	HBMT	TCD	T Cell Replete	HIT	MUDT		ALT	CBIBT	HIT	MSDT
No. of patients	28	25	50	33	32	15	15	21	125	103	40	176
Age, yr, median (range)	45 (19-65)	38.5 (24-57)	24.5 (7-48)	36 (18-56)	45 (20-63)	9.7 ± 1.1	8.6 ± 1.4	45 (29-46)	NA	NA	NA	NA
Male/female sex, n	15/13	17/8	28/22	16/17	19/13	14/1	11/4	NA	NA	NA	NA	NA
Diagnosis	HM	HM	HM	HM	HM + others	AML/ALL	AML/ALL	HM	NA	NA	NA	NA
Myeloablative conditioning	0	Modified Bu/Cy	Modified Bu/Cy + ATG	33	26	0	0	Cy + TBI + Flu	NA	NA	NA	NA
Reduced-intensity conditioning	Flu + Mel + OKT3 + Thi	0	0	0	6	15	15	0	NA	NA	NA	NA
GVHD prophylaxis	MMF	MTX + CSA + MMF	MTX + CSA + MMF	ATG	Cy + Tac + MMF	CSA	CSA	Cy + CSA + MMF	ATG	CSA + MMF	CSA + Cy + MMF	CSA + MTX
Allografts	CD3/CD19-depleted G-PB	G-BM + G-PB	G-BM + G-PB	SS-BM	SS-BM	CD3/CD19-depleted G-PB		SS-BM	SS-BM	SS-BM	SS-BM	SS-BM
Immune recovery, cells/L												
$CD3^+$ T cells												
Day 30 post-SCT	8 at day +40	348	135	NA	NA	870	226	31	146	30	195	477
Day 90 post-SCT	205 at day +100	1048	883	NA	NA	983	652	413 at day +60	404	57	182	565
Day 180 post-SCT	369 at day +400	1037	1163	NA	NA	NA	NA	691	470	196	499	700
$CD3^+CD4^+$ T cells												
Day 30 post-SCT	5 at day +40	141	21	1	26	141	138	11	36	7	45	160
Day 90 post-SCT	70 at day +100	220	152	7	127	305	198	154 at day +60	86	36	127	170
Day 180 post-SCT	177 at day +400	276	163	69	194	NA	NA	193	111	106	211	198
$CD3^+CD8^+$ T cells												
Day 30 post-SCT	2 at day +40	115	98	1	22	730	85	11	102	42	73	280
Day 90 post-SCT	66 at day +100	645	672	17	181	678	447	193 at day +60	278	16	424	389
Day 180 post-SCT	193 at day +400	580	918	54	167	NA	NA	409	413	51	408	500
$CD3^+CD4^+$ naïve cells												
Day 30 post-SCT	NA	90	6	0.7	3.45	3.9	5.3	0.45	NA	NA	NA	NA
Day 90 post-SCT	28 at day +100	152	33	0.55	7.3	3.3	3.3	9 at day +60	NA	NA	NA	NA
Day 180 post-SCT	115 at day +400	196	69	4.8	6.72	NA	NA	24	NA	NA	NA	NA
$CD3^+CD4^+$ memory cells												
Day 30 post-SCT	NA	131	20	2.6	24	136	134	4	NA	NA	NA	NA
Day 90 post-SCT	79 at day +100	155	108	16	97	299	181	65 at day +60	NA	NA	NA	NA
Day 180 post-SCT	205 at day +400	261	171	111	179	NA	NA	48	NA	NA	NA	NA
B cells												
Day 30 post-SCT	NA	13	13	5	0.95	39	24.6	0.5	NA	NA	NA	NA
Day 90 post-SCT	100 at day +150	33	23	55	64	147	43	119 at day +60	NA	NA	NA	NA
Day 180 post-SCT	275 at day +400	60	54	58	95	NA	NA	104	NA	NA	NA	NA
NK cells												
Day 30 post-SCT	248 at day +20	NA	NA	225	84	291	254	59	NA	NA	NA	NA
Day 90 post-SCT	NA	NA	NA	357	183	299	253	189 at day +60	NA	NA	NA	NA
Day 180 post-SCT	NA	NA	NA	290	172	NA	NA	168	NA	NA	NA	NA

HBMT indicates haploidentical blood and BM transplantation; HM, hematologic malignancy; AML, acute myelogenous leukemia; ALL, acute lympoblastic leukemia; ALT, alternative donor (1 antigen-mismatched family or unrelated donors) transplantation; CBIBT, unrelated cord blood grafted intrabone transplantation; NA, not available; Cy, cyclophosphamide; TBI, total body irradiation; Flu, fludarubine; Bu, busulfan; ATG, anti-human thymocyte immunoglobulin; Mel, melphalan; Thi, thiotepa; MMF, mycophenolate mofetil; CSA, cyclosporine A; MTX, methotrexate; Tac, tacrolimus; G-BM, G-CSF–primed BM; G-PB, G-CSF–mobilized peripheral blood stem cell grafts; SS-BM, steady-state BM.

* Data coming from meeting abstract.

Table 2
Effect of Recovered Immune Subsets on Outcomes after Haploidentical Transplantation

Disease (No. of Patients) [Reference]	Allografts	Conditioning Regimen	Reconstituted Immune Subsets	Potential Effects on Clinical Outcomes
Hematologic malignancies (27); other diseases (6) [23]	Selected $CD34^+$ cells	MA (33)	Thymic-dependent T cells: sjTREC <0.1/150,000T TREC <0.001/150,000T	Low thymic function is associated with leukemia relapse.
Hematologic malignancies (30); other diseases (2) [90]	Unmanipulated BM (13) and PBSCs (6); selected $CD34^+$ cells (13)	MA (32)	$CD3^+CD8^+$ T cells	Univariate analysis demonstrated superior survival in patients with $CD8^+CD3^+$ absolute counts above the 5th percentile of age-matched normal levels during the first year post-transplantation.
Hematologic malignancies (43) [24]	G-BM + G-PB	MA (43)	$CD56^{bright}$ NK cells (9.27) and T:NK ratio at day 14 post-transplantation	The patients with more $CD56^{bright}$ NK cells in the recovery stage had a higher survival rate (hazard ratio [HR], 0.406; $P = .017$) and the patients with a T/NK ratio >1.0 had a greater likelihood of getting aGVHD (HR, 3.436; $P = .059$) and chronic GVHD (HR, 3.925; $P = .028$).
Hematologic malignancies (206) [18]	G-BM + G-PB	MA (206)	Early recovered lymphocytes	Multivariate analysis showed that patients with higher day 30 ALC (≥300/μL) was associated with low relapse rate, low TRM, and superior survival in both adult and pediatric patients.
Hematologic malignancies (60) [59]	G-BM + G-PB	MA (60)	Early recovered lymphocytes	
Hematologic malignancies (78) [60]	G-BM + G-PB	MA (78)	Early recovered lymphocytes	
Hematologic malignancies (21) [43]	SS-BM	MA (21)	Early recovered lymphocytes	Patients with a day 30 ALC >200 cells/μL had a markedly improved overall survival and event-free survival.
Hematologic malignancies (22) [33]	G-PB + TCD	MA (22)	iNKT	The frequency of iNKT cells was significantly correlated with remission after transplantation.
Hematologic malignancies (98); nonmalignancies (33) [61]	BM (78); G-PB (42); umbilical cord blood (11)	MA (131)	CMV-specific $CD8^+$ T cells (3 cyt^+ cells/μL); CMV-specific $CD4^+$ T cells (1 cyt^+ cells/μL)	No CMV DNAnemia was evident.
Acute leukemia (30) [41]	G-PB with CD3/CD19 depletion	MA (30)	Lymphoid DC2- cells	A significant correlation was seen between the number of lymphoid $DC2^-$ cells on day +60 with patient survival.
Hematologic malignancies (89) [91]	TCD and TCR allografts	MA (89)	CMV-specific IFN ELISPOT (1000 spots/mL)	Low TRM and virus reactivation were noted.
Hematologic malignancies (47) [63]	Unmanipulated PBSC (27); BM (20)	Nonmyeloablative (30); MA (17)	Treg:$CD4^+$ T cell ratio at day 14 post-transplantation	Multivariate analysis showed that a cutoff ratio value of 9% yielded the most accurate predictions of future aGVHD incidence. Treg:$CD4^+$ T cell ratios of <9% predicted a significantly higher incidence of aGVHD compared with ratios ≥9% ($P = .0082$).

PBSCs indicate peripheral blood stem cells.

and leukemia relapse [2,3,71,87,88]. These results underscore the critical importance of improving immune recovery in both TCD and T cell–replete haploidentical HSCT settings.

ASSOCIATION OF RECOVERED IMMUNE CELLS WITH TRANSPLANTATION OUTCOMES

A number of studies have evaluated the association of immune recovery with clinical outcomes after haploidentical transplantation (Table 2) [18,24,59,60]. A higher absolute lymphocyte count at day 30 post-transplantation (ALC-30; ≥300 cells/μL) is associated with lower incidences of relapse and transplantation-related mortality (TRM) and superior leukemia-free survival and overall survival in both adult and pediatric patients after unmanipulated haploidentical blood and BM transplantation [18,59,60]. Using the unmanipulated haploidentical HSCT protocol, the Peking University group demonstrated a survival advantage for patients with high early (day +14) levels of $CD56^{bright}$ NK cells after unmanipulated haploidentical HSCT [24]. In Spain, Martin et al. [89] confirmed this result using unmanipulated haploidentical BM grafts after RIC. Other factors, including lower numbers of recovered $CD3^+CD8^+$ T cells and lymphoid mDC2 cells, may contribute to inferior survival [41-90]. Fujioka et al. [63] reported that a Treg:$CD4^+$ T cell ratio <9% is predictive a significantly higher incidence of acute GVHD compared with a ratio ≥9% ($P = .0082$) after unmanipulated blood or BM transplantation. This finding awaits confirmation in other haploidentical HSCT protocols, such as transplantation with CD3/CD19-depleted allografts [8] and BM transplantation with high-dose post-transplantation cyclophosphamide [71].

Another study investigated multiple parameters of T cell immune reconstitution in 89 patients who underwent haploidentical HSCT. Using a receiver operating characteristic curve analysis of cytomegalovirus (CMV)-specific IFN enzyme-linked immunosorbent spot (ELISPOT) assay results at day 30 to 90, Noviello et al. [91] found that cutoff values of 1000 spots/mL allowed researchers to identify those patients

who did not reactivate the virus at later time points with high specificity (>95%). Strikingly, the 2-year TRM rate was 32% in patients with an ELISPOT finding of <1000 spots/mL and 0% in those with >1000 spots/mL ($P < .05$). This interesting finding warrants the use of a predictive biomarker in larger multicenter series to investigate whether a CMV-specific IFN- ELISPOT cutoff value of 1000 spots/mL is a strong surrogate biomarker for TRM. When Clave et al. [23] evaluated thymic function in a group of pediatric recipients, they found that 6 months after CD34-selected HLA-haploidentical HSCT, a TREC value below the limit of detection (<0.1 per 150,000 $CD3^+$ T cells for sjTREC and <0.001 per 150,000 $CD3^+$ T cells for TREC) was associated with an increased risk of relapse [23].

In summary, prospective studies with larger patient populations are needed to draw definitive conclusions regarding the impact of recovered immune cells on clinical outcomes in T cell–replete and TCD nonmyeloablative or myeloablative haploidentical settings [3,8,92]. These studies are important to the prediction of such clinical outcomes as infection rates, relapse, and TRM [37,62]. Shimoni [62] suggested that the ALC-30 has long been a simple test of immune recovery that can predict outcome after autologous HSCT as well as allogeneic and haploidentical HSCT [18,59,60]. It is easy, reproducible, and requires no special expertise. Thus, the measurement of ALC-30 may have practical significance in applying interventions to facilitate immune reconstitution as described previously for haploidentical transplants [18,42,59,60].

COMPARISION OF IMMUNE RECOVERY BETWEEN HAPLODENTICAL AND OTHER TRANSPLANTATION MODALITIES OR GRAFT OPTIONS

Several published prospective and retrospective studies have highlighted the differences in immune reconstitution after unmanipulated haploidentical HSCT versus HLA-matched sibling donor transplantation (MSDT), matched unrelated donor transplantation (MUDT), or umbilical cord blood transplantation (Table 1). Chang et al. [25] found that compared with HLA-matched recipients, haploidentical recipients had lower counts of T cells (particularly CD4 T cells) and DC subsets before day +90, as well as greater CD28 expression on $CD4^+$ and $CD8^+$ T cells, whereas T cells were equally functional in the 2 patient groups at day +30. In addition, Dominietto et al. [93] recently demonstrated early delayed immune reconstitution after haploidentical transplantation (HIT) compared with MSDT [93]. Several lines of evidence suggest an association between improved immune recovery and better transplantation outcomes [13,24,94-96]; however, in our center, transplantation outcomes regarding overall survival and leukemia-free survival were comparable after HIT and MSDT, although unmanipulated HIT was characterized by delayed early reconstitution of T cells and DCs, as well as a higher incidence of CMV antigenemia [86,87]. Several factors may account for this finding. Compensatory expansion of monocytes, NK cells, and cytotoxic T lymphocytes (CTLs) [25], especially CMV-pp65 peptide-specific CTLs with the central memory $CD45RO^+CD62L^+$ cell phenotype, accompanies the recovery of $CD8^+$ T cells. This cell population may proliferate and differentiate into effector memory T cells stimulated with CMV antigen and may contribute to a reduced incidence of CMV disease [31,47]. Other possible contributing factors include the preemptive management of CMV antigenemia, a superior graft-versus-leukemia effect associated with transplantation of haploidentical versus HLA-identical sibling donor grafts for high-risk acute leukemia [97], and improvements in minimal residual disease–directed relapse prophylaxis using modified donor lymphocyte infusion [66,98-100].

In a detailed prospective analysis of immune reconstitution in MUDT and HIT with CD3/CD19-depleted grafts in 30 pediatric patients, Pérez-Martínez et al. [41] found more rapid CD8 and CD8 memory T cell recovery during the first month in HIT compared with MUDT (730 ± 208 versus 85 ± 37 cells/mL and 723 ± 274 versus 83 ± 29 cells/mL, respectively; $P < .05$). CD80 expression in the DC1-DC2- subset was higher in HIT patients than the CD80 expression in the DC1-DC2- subset in MUDT patients during the first 2 months after HSCT (respectively, 26 ± 6.98 vs 5 ± 1.4/mL, $P = .04$ and 28 ± 12 vs 2 ± 0.8/mL, $P = .05$); however, the reconstitution of NKT cells, NK cells, and B cells was comparable in the 2 groups [41]. Szabolcs et al. [101] reported an association between $CD8^+$ lymphocytosis and effector memory phenotype and IFN secretion in children who developed de novo opportunistic infections. Brown et al. [102] presented data on recent thymic emigrants and CMV-specific immune responses demonstrating associations between higher TREC and CMV-specific immune responses and improved survival. Sauter et al. [103] found that an ATG-free regimen after double cord blood transplantation could result in more rapid $CD4^+$ T lymphocyte reconstitution, and reported no infection-related deaths after day +120, suggesting a correlation between immune recovery and infection-related death. Continuing new insights into the mechanisms underlying differences in the kinetics of immune reconstitution after HIT compared with MSDT and MUDT may help guide the design of useful strategies to accelerate immune recovery in HIT settings.

STRATEGIES TO IMPROVE IMMUNE RECONSTITUTION AFTER HAPLOIDENTICAL HSCT

Many of the currently available approaches to accelerating immune recovery, including IL-7, keratinocyte growth factor, growth hormone, and adoptive transfer of immune cell subsets, have been reviewed elsewhere [12,19,37,66,104-106]. Here we discuss only recent informative updated trials and reports (Table 3); data reported between 2012 and 2013) regarding the promotion of immune reconstitution, particularly in haploidentical transplantation settings [53,65,69,107].

Cytokines

IL-7

Among the cytokines used to enhance T cell reconstitution, IL-7 certainly tops the list. In a phase 1 trial, Perales et al. [65] treated 12 patients who underwent MSDT or MUDT with escalating weekly doses of IL-7 for a 3-week period. Three patients received 10 μg/kg, 6 patients received 20 μg/kg, and 3 patients received 30 μg/kg. IL-7 was well tolerated overall, with only 1 patient developing acute skin GVHD. Compared with baseline values, levels of recovered $CD3^+$, $CD4^+$, and $CD8^+$ T cells increased by 4.3-, 6.1-, and 4.3-fold, respectively. IL-7 also enhanced functional T cell responses and TCR diversity. Unfortunately, IL-7 appeared to have no significant effect on $CD4^+CD25^+FoxP3^+$ T cells, NK cells, or B cells, although the data that IL-7 significantly enhances T cell recovery are exciting [65]. Based on the foregoing findings, other approaches applied alone or in combination with IL-7 to promote the reconstitution of all immune subsets merit investigation in both HLA-matched and haploidentical transplantation protocols [2,3,87,88].

Table 3
Recent Informative* Trials and Reports Regarding Strategies for Enhancing Immune Recovery

Strategy	Transplantation Protocol	Key Points	Reference
Approaches used in preclinical model			
IL-15	HIT mice model	IL-15 can be used for enhancing antileukemia effect of haploidentical HSCT, which requires presence of donor-derived T cells, especially $CD8^+$ T cells.	Sauter et al. 2013 [58]
Depletion of naive lymphocyte with Fas ligand	HIT mice model	The proposed approach eliminates severe GVHD in T cell–replete transplants, preserves the tumor lytic activity of mismatched donor T cells, maintains T cell reactivity to third-party antigens, and, facilitates HSC engraftment.	Askenasy et al. 2013 [49]
Approaches used in clinical trial or report			
IL-7	MRDT and MUDT	Treatment 12 patients with IL-7 increases T cell recovery, induces T cell responses, and enhances TCR diversity. IL-7 have no significant effect on Treg, NK or B cells.	Perales et al. 2012 [65]
Escalating-dose DLI	HIT	This study suggests that DLI, using an excalating-dose regimen, can be safely given to mismatched transplant recipients with a high rate of molecular remission and a low risk of GVHD.	Innes et al. 2013 [55]
Modified DLI	HIT and MRDT	Eight hundred and fourteen patients were enrolled, this study suggest that risk stratification-directed modified DLI may reduce relapse and improve survival of subjects with standard-risk acute leukemia after HSCT, although the effect of DLI on immune reconstitution need to be investigated.	Yan et al. 2012 [98]
Preemptive immunotherapy with NK cells	HIT	In this prospective phase II study, the results suggest that NK cell activation/expansion may be required to attain clinical benefit, while careful consideration must be given to the number of T cells infused.	Stern et al. 2013 [108]
Adoptive transfer of suicide gene-tranduced donor T cells	HIT	Thymopoiesis is reactivated after the infusion of gene-modified lymphocytes, enhancing the generation of a protective T cell compartment that is able to reduce the incidence of infectious and control of GVHD.	Vaglo et al. 2012 [65]
Adoptive transfer of donor-derived CMV-specific T cells	HIT, MSDT, and MUDT	Infusion of CMV-specific T cell early posttransplant reduced the need for pharmacotherapy without increased rates of CMV-related organ damage and increasing acute and chronic GVHD.	Blyth et al. 2012 [69]
Adoptive transfer of trivirus-directed T cells	HIT, MSDT, and MUDT	In ten patients, trivirus-directed T cells administration produced completed virological responses in 80% cases and a decrease in viral load was associated with an increase in the frequency of T cell directed against the infecting virus. No toxicities were observed.	Gerdemman et al. 2013 [63]

MRDT indicates HLA-matched related donor transplantation; DLI, donor lymphocyte infusion.
* Published between 2012 and 2013.

IL-15

Using a clinically relevant haploidentical murine transplantation model, Sauter et al. [58] found that IL-15 administration significantly increased $CD8^+$ T cell and NK cell reconstitution and also improved graft-versus-leukemia/graft-versus-tumor activity in recipients of haploidentical HSCT. These authors also demonstrated that IL-15 increased intracellular IFN secretion in undivided and slowly proliferating $CD8^+$ T cells in recipients of carboxyfluoresceinsuccinimidyl ester–labeled T cells, with no change in the IFN-secreting alloreactive/rapidly proliferative cell population [58].

The efficiency, safety, and feasibility of using IL-15–stimulated CD3/CD19-depleted stem cells or IL-15–activated cytokine-induced killer cells to treat relapse after haploidentical transplantation have been investigated, albeit only in small patient populations [51,57]. Pfeiffer et al. [57] reported that in 8 patients, recovered $CD56^+$, $CD3^+/CD4^+$, and $CD3^+/CD8^+$ lymphocyte counts at 15 to 30 days after receipt of IL-15–stimulated stem cell boosts were similar to or even higher than those before the initiation of chemotherapy.

Preemptive Transfer of NK Cells

Adoptive immunotherapy with allogeneic purified NK cell products might exert graft-versus-tumor alloreactivity with little risk of GVHD. In a prospective phase 2 study, Stern et al. [108] administered purified NK cell products to high-risk patients treated with haploidentical TCD HSCT. Sixteen patients received a total of 29 NK cell infusions on days +3, +40, and +100 post-transplantation. The median dose of infused NK cells per product was 1.21×10^7/kg (range, 0.3-3.8 $\times$ 10^7/kg). Four of the 16 patients were alive at the time of this report, at a median follow-up of 5.8 years. Rapid reconstitution of lymphocyte subsets at day +100 was observed, with a median CD8 T cell count of 266 cells/μL and a median CD4 T cell count of 181 cells/μL. These promising results warrant further study to determine the optimal dose and timing of NK cell immunotherapy for antileukemia activity.

Genetically Modified Donor T Cell Infusion

Several researchers have investigated the use of suicide gene–transduced donor T cells to enhance immune reconstitution while controlling GVHD. In a multicenter phase 1/2 clinical trial, purified herpes simplex thymidine kinase suicide gene–transduced donor T cells (TK^{pos} cells) were infused serially into 28 adult patients with a hematologic malignancy after CD34-selected haploidentical transplantation [70]. Twenty-two of the 28 patients who received the purified TK^{pos} cells exhibited a rapid recovery of T cells associated with a concomitant improvement in clinical outcomes and reduced TRM. Patients who did not receive TK^{pos}

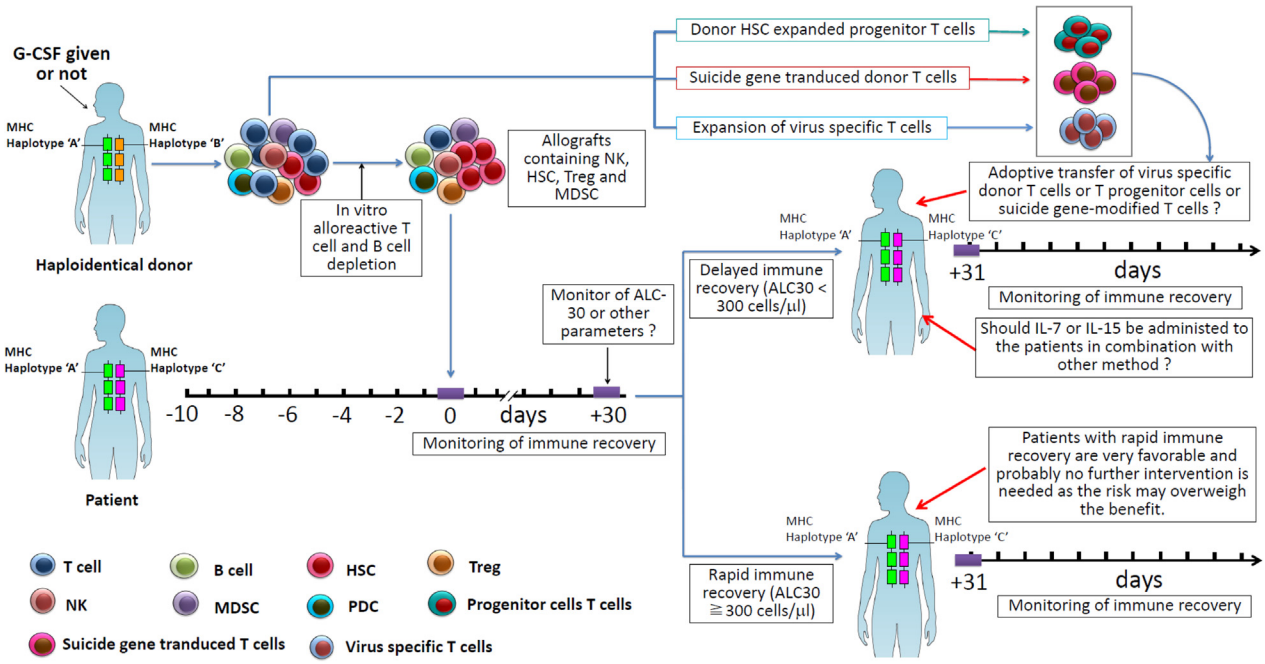

Figure 1. A proposed prognostic immune subset–directed strategy to improve immune reconstitution after HIT. On day 0, in vitro alloreactive T cell–and/or B cell–depleted allografts, including NK cells, HSCs, Tregs, and myeloid-derived suppressor cells (MDSCs), were infused to the recipients who received a conditioning regimen to decrease the incidence of GVHD and enhance immune reconstitution. After transplantation, the kinetics of immune recovery were monitored. For patients with rapid immune recovery (as demonstrated by ALC-30 $\geq$ 300/μL or another parameter predictive of rapid immune recovery), no interventions were required, and immune reconstitution was monitored only. For patients with delayed immune recovery (ALC-30 < 300/μL or other parameters predictive of delayed immune recovery), interventions, including IL-7 and/or adoptive transfer of expanded progenitor T cells or virus-specific donor T cells, were implemented to improve immune reconstitution. MHC, major histocompatibility complex.

cells failed to attain T cell immune reconstitution and experienced dismal outcomes, owing mainly to infectious complications. Impressively, further follow-up of these patients revealed that transfer of TK^{pos} cells not only enhanced the regeneration of $CD3^+$ T cells, but also increased counts of the $CD4^+$ T cell subset and the T cell subset with a naive phenotype ($CD62L^+CD45RA^+CD31^+$), as well as sjTREC counts, in parallel to the increase in the naive T cell subset. Strikingly, an increase in serum IL-7 level was observed early after the TK^{pos} T cell add-back, followed by a concomitant rise in peripheral T cell counts. Computed tomography–based analysis revealed an increase in thymic tissue even in elderly patients. Additional experiments demonstrated that the antiviral activity of the newly generated T cells was fully maintained.

The biological mechanism responsible for these unexpected findings is unclear. The transient increase in IL-7 might be a contributing factor, as described by Perales et al. [65] in a phase 1 trial, but is likely insufficient to induce long-lasting thymopoiesis. The findings of Vago et al. [70] might suggest new approaches to accelerating immune reconstitution in patients not only after haploidentical transplantation of $CD34^+$ stem cells, but also in other transplantation settings, and particularly in elderly patients [2,3,67,71,88].

Adoptive Transfer of Pathogen-Specific T Cells

Gerdemann et al. [49] recently developed a new rapid and simplified manufacturing strategy in which DCs nucleofected with DNA plasmids encoding a range of immunodominant and subdominant viral antigens from adenovirus (Adv), CMV, or Epstein-Barr virus (EBV) are used to generate trivirus-specific T cells. In a recent study, trivirus-directed T cells were administered to recipients of haploidentical (n = 5), matched unrelated (n = 3), mismatched unrelated (n = 1), or matched related (n = 1) transplants with active CMV (n = 3), Adv (n = 1), EBV (n = 2), EBV + Adv (n = 2), or CMV + Adv (n = 2) infections. The cells produced complete virologic responses in 80% of recipients, including all patients with dual infections. In each case, a decrease in viral load was correlated with an increase in the frequency of T cells directed against the infecting virus(es); both immediate and delayed toxicities were absent [68]. The authors suggested that a DNA plasmid-based approach might increase both the feasibility and applicability of T cell therapy.

Little data are available on vaccine responses in patients undergoing HIT [109]. More recently, several other approaches, including the depletion of naive lymphocytes using Fas ligand [53] and the infusion of $CD62L^-$ memory T cells [107], have been used in preclinical models to enhance immune reconstitution after transplantation. Additional studies are warranted to investigate whether these preclinical approaches can be translated into clinical trials.

FUTURE DIRECTIONS

Delayed immune reconstitution is an important issue in haploidentical HSCT. Substantial progress has been made in the field, including insight into recovered immune subsets that can predict outcomes and new strategies for accelerating immune recovery. These advances make it possible to establish a prognostic immune subset–directed strategy to improve immune recovery in haploidentical settings (Figure 1). To achieve this goal, prospective, multicenter trials are needed to investigate whether a low ALC-30 or other simple immune parameters can be used to identify which patients should be treated to improve immune recovery. In

addition, pilot studies should be conducted to evaluate the most effective immune reconstitution-accelerating strategy or combination of approaches. Although promising survival has been achieved with the establishment of many haploidentical transplantation protocols, we believe that a predictive immune subset–guided strategy to accelerate immune recovery might further improve transplantation outcomes in the future, making haploidentical transplantation a routine strategy for patients requiring HSCT.

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